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DOCTORAL SCHOOL OF HEALTH SCIENCES

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The Relationships Between Extreme Physical and Mental Stress and Endogenous Steroid Biosynthesis in Professional Athletes

Doctoral thesis summary

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1. Introduction

The regulation mechanisms activated by physical activity, sports, and especially elite sports, play a significant role alongside neural regulation, with notable importance attributed to endocrine regulatory mechanisms. Among these, the functioning of the adrenal glands is of paramount importance, as cortical steroid hormones are involved in the regulation of metabolism, the salt and water homeostasis, and, in the case of male athletes, their sexual functions and physical performance. Of these, cortisol (CTOL), aldosterone (ALDO), and testosterone (TEST) have been studied most extensively, yet all three steroid branches are associated with related effects. Therefore, studying the effects of mental and physical stress in full or partial isolation based on models that contain either mental or physical components on the steroid homeostasis conveys important information.

The analysis of steroid hormones plays a significant role in diagnosing athlete overtraining and exhaustion, as well as in the characterization of the impact of steroids of exogeneous origin, both licit and illicit, on endogenous steroid synthesis. In addition, it is important to see how steroid levels change during peak athletic performance. When characterizing the endocrine status of elite athletes, it is important to consider that taking blood samples for assaying each relevant parameter is not possible due to the associated loss of red blood cells and hemoglobin. At the same time, characterizing steroid metabolism using a complex approach requiring a single sample can bring us closer to understanding the bidirectional relationship between muscle function and the adrenal function.

Steroid profiling has been employed for the diagnosis of various clinical conditions, but such measurements have been lacking in healthy athletes. The aim of the research was to develop and apply a methodology for the assessment of adrenocortical and gonadal steroid levels in a single sample collected from athletes under resting conditions, as well as during maximum physical and mental stress in elite athletes.

2. Objectives

The primary aim of my research was to investigate whether a steroid profiling method could be applied for studying the changes of the adrenocortical and gonadal functions of healthy elite athletes in the resting state as well as under physical or mental stress. My next aim was to learn whether metabolite molecules providing similar responses to those already known to increase in concentration in response to stress could be identified. Further, I aimed to investigate whether the responses given to a 2-hour long exercise were different from those observed during an acute spiroergometric exercise. Finally, my aim was to reveal whether the hormonal response given to mental stress models was different from that given to physical stress.

In the framework of my research, I examined the changes in the concentrations of 16 different endogenous steroid hormones in male athletes in response to exposure to extreme physical and mental stress. The following objectives were set in these experiments:

- The simultaneous determination of a large number of steroid metabolites in a single serum sample in the field of exercise physiology for the examination of male elite athletes.
- Characterization of the changes in the concentrations of circulating steroid metabolites in male elite athletes in response to extreme physical stress, using both the acute "vita maxima" type and long-term 2-hour protocols.
- Examination of the changes in the concentrations of circulating steroid metabolites in male elite athletes in a mental stress-model.

3. Materials and methods

3.1. Participants

Healthy Caucasian male adult elite athletes from Hungary were recruited. The participants were self-reportedly non-smokers, and did not have any known cardiovascular disease. The study participants formed three groups:

- **extreme physical and extreme mental load test group:**
    - o "EFM" group: (n=40) elite handball athletes (median age: 22 years, IQR: 20–25 years). Number of hours of weekly training for the participants: 21 hours.
- **preliminary physical load test group:**
    - o "ET" group (n=6): elite kayak-canoe athletes (median age: 20 years, IQR: 18-23 years)
    - o Control group (n=6): untrained persons (median age: 23 years, IQR: 19-26 years)

3.2. Load protocols

Extreme physical load protocol of elite athletes

The physical stress test was executed by applying the modified Bruce protocol (2-min. warm-up at 8 km/h speed, then increased to 10 km/h and kept constant, and an elevation of 0% kept for 3 min and subsequently increased by 1.5%/min) in an exercise physiology laboratory (University of Physical Education, Department of Health Sciences and Sports Medicine, Budapest, Hungary), where the participants underwent an ergospirometry treadmill test, reaching their maximal voluntary exhaustion.

Extreme mental load protocol of elite athletes

The extreme mental stress test was conducted independently from the physical exercise. The protocol was developed with the participation of a clinical psychologist expert at the International Training Center of the Ministry of Interior (Budapest, Hungary). We applied a modified, less complex version of the original protocol. Athletes were exposed to stress in a specialized psychological tactics room. Participants were provided with protective gear and simulator weapons, along with

detailed information and preparation, 10 minutes before entering the psychological tactics room. Participants entered the room and had to proceed through it. During their stay inside, they were exposed to stress-inducing factors (e.g., sudden appearance of a person). The peak of the stress was marked by the appearance of an organizer "attacker" who fired two times with blank rounds towards the participant. The duration of stay in the psychological tactics room for athletes ranged from 1 to 4 minutes.

Acute load protocol

The participants were subjected to acute, voluntary exhaustion using continuous spiroergometry on a recumbent bicycle ergometer. The pedal speed of the ergometer was set at 70-80 revolutions per minute, with an increase with 50-watt increments every 3 minutes until complete exhaustion. Data recording and sampling were performed at rest, during the aerobic phase (RQ=0.9), upon reaching the anaerobic threshold (RQ=1.0), following the peak of the exertion, and at the 5^{th} and 30^{th} minutes of restitution phase.

Long term load protocol

We conducted prolonged, 120-minute-long exercise with the participation of six athletes using a recumbent bicycle ergometer with spiroergometry, where the subjects continuously exercised for 120 minutes. Every 20 minutes, we interrupted the exercise for approximately 1 minute to collect blood samples, and to record data. After 120 minutes, we intensified the exercise, increasing it by 50 watts every 3 minutes until complete exhaustion.

Blood samples were collected using a closed blood sampling technique before the exercise in a resting state, at the point of reaching maximum exertion, and 30 minutes after completing the exercise. The samples were centrifuged, and the supernatants were aspirated, flash-frozen with liquid nitrogen, then stored at -80°C until performing the measurements.

3.3. Serum steroid profile analysis

Steroid concentrations were measured at the Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary using a validated in-house liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. A Shimadzu Nexera X2 ultra-high performance liquid chromatograph was coupled to a Shimadzu LCMS-8060 triple quadrupole mass spectrometer. Instrument control and data acquisition were attained with the Shimadzu LabSolutions MS 5.89 software (Simkon Kft., Budapest, Hun-gary). For separation, a combination of Phenomenex Kinetex XB-C18 and biphenyl analytical columns (dimensions: 50x2.1 mm for both stationary phases, particle size: 1.7 μm), thermostatted at 40°C, was used. The mobile phase consisted of water-0.1% formic acid (A) and methanol-0.1 % formic acid (B). The run time was 5.5 min, and the injection volume was 5 μl. The measured steroids were aldosterone (ALDO); androstenedione (ADRN); 11-deoxycortisol (11DC); dehydroepiandrosterone (DHEA); dehydroepiandrosterone sulfate (DHES); dihydrotestosterone; cortisol (CTOL); cortisone (CTON); corticosterone (CCON); 17-α-hydroxypregnenolone (OHPE); 17-α-hydroxyprogesterone (OHPG); testosterone (TEST).

Basic calculations were performed using Microsoft 365 Excel. Multivariate statistical analysis was conducted using the MetaboAnalyst online platform (version 5.0, https://www.metaboanalyst.ca/) DCON and 21DC were omitted from the statistical evaluation, because in most cases, their concentrations were below the detection limit. Further, a specific feature was removed when more than 25% of the measured concentrations were lower than the limit of detection. In order to approximate a normal distribution, data were processed following glog transformation and autoscaling. The exploratory analysis was conducted using principal component analysis (PCA), nonparametric analysis of variance (Kruskal-Wallis test) and Spearman's correlation test. Univariate statistical analysis was performed employing IBM SPSS Statistics version 25. with Mann-Whitney and Wilcoxon singed-rank test. $P \leq 0.05$ was considered as the threshold of a statistically significant difference.

4. Results

4.1. Performance of extreme physical and mental stress tests

All selected participants completed the stress tests successfully. No injuries or discomfort were observed, and there was no need to repeat any of the tests for technical or other reasons. During the examination of the EFM group, the physiological parameters monitored confirmed that the participants had been exposed to extreme physical stress. In the case of mental stress, heart rate and blood pressure values changed in most cases similarly to physical stress.

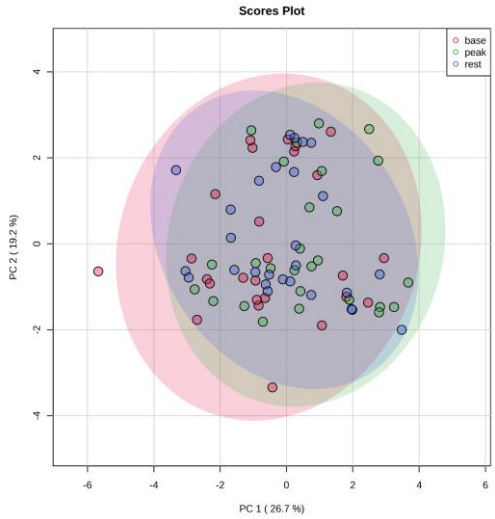
The criteria established for acute extreme physical stress did not apply to prolonged stress. At the peak of the stress, the lactate concentration was lower than 8 mmol/L in four of the six participants, and the median respiratory quotient (RQ) value was lower than 1.1 in five participants. In the case of acute stress, heart rate (HR), systolic blood pressure (SYS), and lactate concentrations changed in the same direction, showing a significant increase compared to the resting values at the peak of the stress, and a significant decrease during the restitution phase compared to the peak measurement. These values were significantly higher in the restitution phase than the resting values. In the ET group, during prolonged stress, HR and lactate concentration showed similar changes as in acute stress. HR and lactate concentration significantly increased at the maximum stress point compared to resting values, then decreased during the restitution phase but remained significantly higher than baseline levels.

In the control group, the criteria for physical stress were met at all points during acute stress as well. Heart rate and lactate concentration significantly increased at the peak of the stress compared to resting values, and during the restitution phase, they decreased, but remained significantly higher than baseline values.

4.2. Results of the EFM group's extreme physical and extreme mental stress-physiological tests

Based on the results of principal component analysis (PCA) (Figure 1), no single steroid or steroid group accounted for the differences among the results obtained in the three load phases.

A



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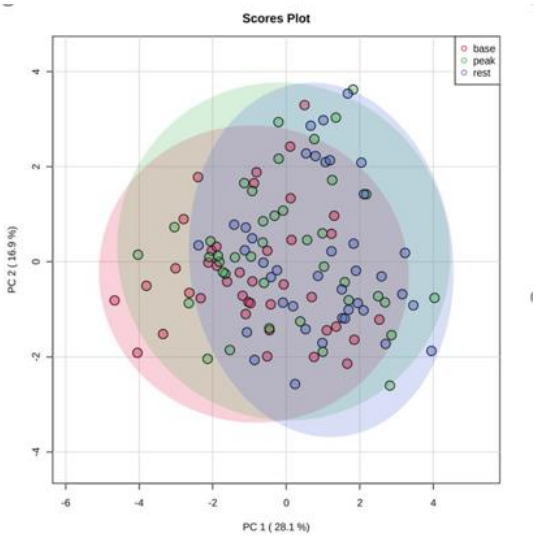


Figure 1: Principal component analysis score plot of glog-transformed steroid levels of the elite handball athletes (EFM) group under mental (A) and physical (B) stress (n=40). In the figures, the concentrations measured in each load phase are represented separately.

These results were confirmed by Kruskal–Wallis tests (Figure 2). However, within the categories of mineralocorticoids (ALDO and CCON), glucocorticoids (CTON and CTOL), and androgens (DHEA, TEST), we identified hormones that exhibited significant changes. In the case of mental stress, no steroids showed significant changes.

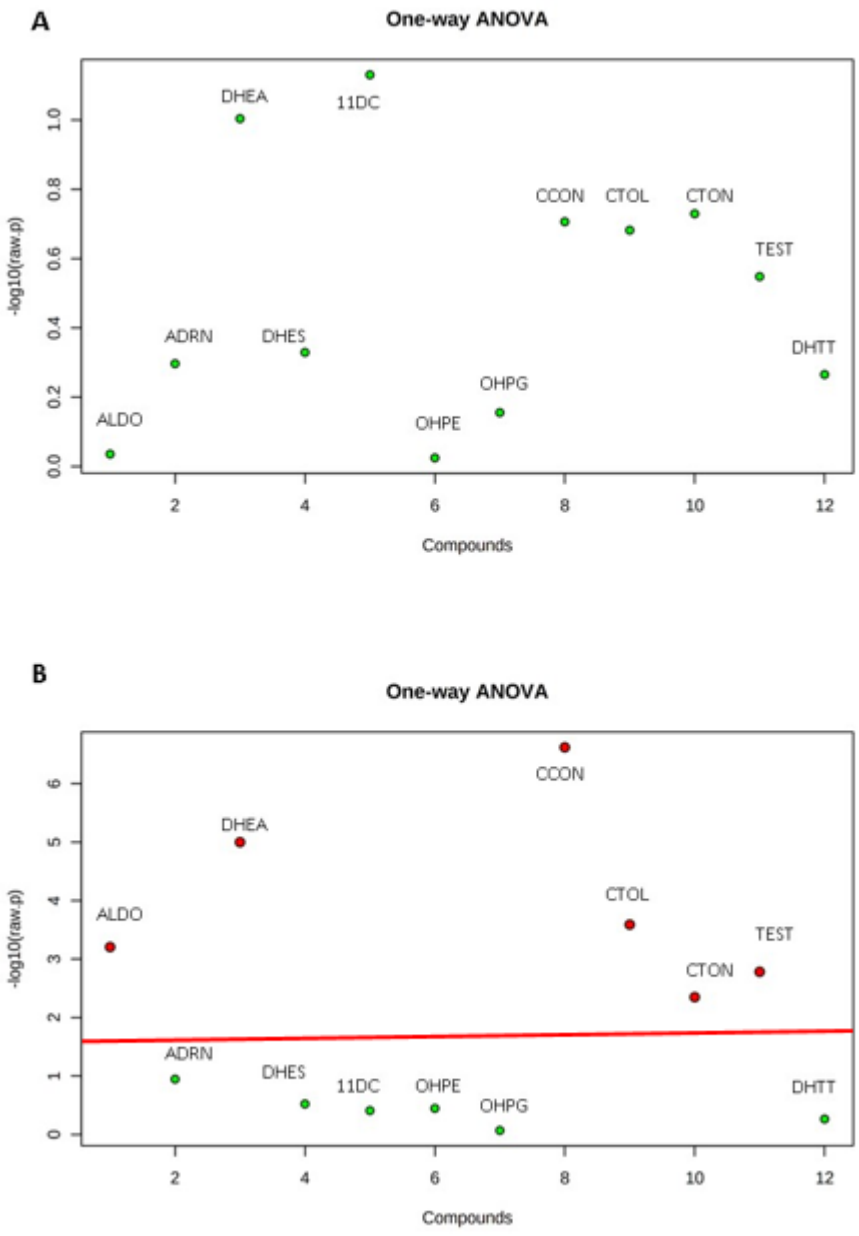


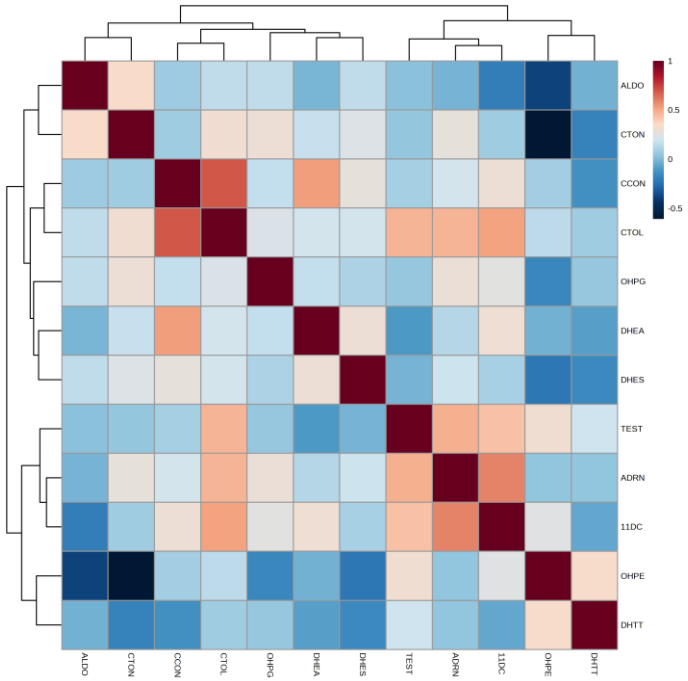
Figure 2: The results of the non-parametric analysis of variance of the Glog-transformed steroid concentrations of the elite handball athletes (EFM) group in the case of (A) mental and (B) physical load (n=40). The red line indicates the threshold p=0.05. ALDO: aldosterone; ADRN: androstenedione; 11DC: 11-deoxycortisol; DHEA: dehydro-epiandrosterone; DHES: dehydroepiandrosterone sulfate; DHTT: dihydrotestosterone; CTOL: cortisol; CTON: cortisone; CCON: corticosterone; OHPE: 17-α-hydroxypregnenolone; OHPG: 17-α-hydroxyprogesterone; TEST: testosterone.

Using Spearman's correlation analysis (Figure 3) based on the Evans categories, we found strong correlations under extreme physical stress between ADRN and 11DC (r=0.65), as well as between CCON and CTOL concentrations (r=0.72). We observed moderate correlations between ADRN and DHEA (r=0.52), DHEA and CCON (r=0.58), CTON and DHEA (r=0.41), DHES and DHEA (r=0.41), DHEA and CTOL

(r=0.42), ADRN and DHTT (r=0.42), and between 11DC and CTOL concentrations (r=0.44) during extreme physical stress.

In the case of extreme mental stress, based on the Evans categories, we only observed a strong correlation between CCON and CTOL (r=0.69), while in most cases, similar to extreme physical stress, we found only moderate correlations: CCON and DHEA (r=0.53), CTOL and ADRN (r=0.47), CTOL and 11DC (r=0.52), CTOL and TEST (r=0.47), ADRN and TEST (r=0.48), TEST and 11DC (r=0.43), and ADRN and 11DC (r=0.59).

A



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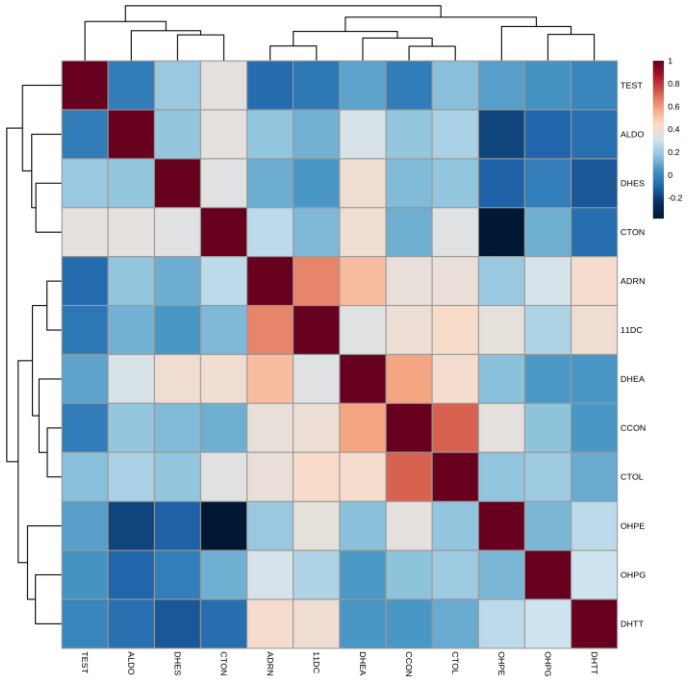

Figure 3: The heat-map of the Spearman's correlation analysis of the Glog-transformed concentrations of steroids measured in elite handball athletes (EFM) group in the case of (A) mental and (B) physical load (n=40). In the two heatmaps, the strength of correlation between individual steroid hormones is depicted, ranging from blue to red. ALDO: aldosterone; ADRN: androstenedione; 11DC: 11-deoxycortisol; DHEA: dehydroepiandrosterone; DHES: dehydroepiandrosterone sulfate; DHTT: dihydrotestosterone; CTOL: cortisol; CTON: cortisone; CCON: corticosterone; OHPE: 17-α-hydroxypregnenolone; OHPG: 17-α-hydroxyprogesterone; TEST: testosterone.

For the non-parametric univariate statistical analysis of the EFM group, we applied the Wilcoxon singed-rank test to compare the measured, non-transformed steroid concentrations in different stress phases. Under extreme physical stress, in most cases, steroid concentrations significantly increased from baseline to the stress peak, and decreased in the restitution phase compared to peak stress (Table 1, 2). ALDO, ADRN, OHPG, CTON, TEST, and DHTT concentrations increased, while CTOL concentrations significantly decreased from baseline to the peak. Compared to the concentrations measured at peak stress, ALDO, DHEA, OHPE, CCON, and CTOL significantly increased, while DHES, OHPG, CTON, and TEST concentrations significantly decreased in the restitution phase. After physical stress, significantly higher concentrations were observed for all steroids compared to the baseline values, except for OHPG.

We applied the Wilcoxon test not only for individual steroids in the group of the 40 participants, but also for concentration ratios that could be relevant for characterizing physical stress. TEST/CTOL, DHEA/CTOL, CCON/CTOL, CCON/DHEA. TEST/CTOL significantly increased from baseline to the stress peak and significantly decreased from the peak stress peak to restitution. DHEA/CTOL levels consistently increased from baseline to the peak stress peak and from the peak stress peak to restitution. CCON/CTOL and CCON/DHEA were significantly higher in restitution compared to the peak stress peak.

In contrast, during extreme mental stress in the EFM group, most steroid hormone levels significantly increased at the peak of stress peak compared to the baseline (ADRN, DHES, 11DC, CTOL, TEST). Only CCON hormone levels significantly increased during the restitution phase compared to the baseline. Compared to the maximum value, during the restitution phase, the concentration of steroids showing significant changes (ADRN, DHES, 11DC, OHPE, CTON, TEST, DHTT) significantly decreased.

Table 1: Non-parametric univariate statistical comparison (Wilcoxon test) of endogenous steroid concentrations measured in the elite handball athletes (EFM) group before exercise, at maximum exercise and after exercise in the restitution phase during extreme mental exercise (n=40). Median values, interquartile ranges, the direction of the changes and the statistical significance of the differences are shown. ↑: increase; ↓: decrease; *: p≤0.05; **: p≤0.01; ***: p≤0.001. ALDO: aldosterone; ADRN: androstenedione; DHEA: dehydroepiandrosterone; DHES: dehydroepiandrosterone sulfate; 11DC: 11-deoxycortisol; OHPE: 17-α-hydroxypregnenolone; OHPG: 17-α-hydroxyprogesterone; CCON: corticosterone; CTOL: cortisol; CTON: cortisone; TEST: testosterone; DHTT: dihydrotestosterone.

	Baseline-maximum	Baseline-restitution	Maximum-restitution	pmol/mL		
	Response			Baseline	Maximum	Restitution
ALDO				0,083 (0,083-0,182)	0,083 (0,083-0,138)	0,083 (0,083-0,160)
ADRN	↑ **		↓ *	1,39 (0,90-1,82)	1,69 (1,10-2,38)	1,49 (0,97-1,89)
DHEA				6,12 (3,71-12,80)	9,93 (5,41-12,58)	8,79 (5,72-13,68)
DHES	↑ *		↓ **	7002 (5021-9044)	7924 (5713-10089)	7015 (5088-8623)
11DC	↑ **		↓ *	0,14 (0,14-0,14)	0,14 (0,14-0,55)	0,14 (0,14-0,21)
OHPE			↓ *	1,95 (0,00-3,93)	2,55 (0,00-4,22)	1,80 (0,00-4,31)
OHPG				0,97 (0,00-2,50)	1,30 (0,00-4,12)	1,15 (0,00-3,12)
CCON		↑ *		6,22 (3,69-10,66)	9,55 (4,30-14,40)	9,84 (4,92-14,30)
CTOL	↑ **			344 (253-441)	390 (303-530)	357 (304-452)
CTON			↓ **	69,8 (61,4-89,3)	79,6 (66,7-101)	72,3 (68,2-90,6)
TEST	↑ *		↓ *	16,7 (13,1-21,0)	18,6 (15,0-21,9)	17,6 (14,9-21,6)
DHTT			↓ *	1,89 (0,91-3,22)	2,02 (1,17-3,48)	1,88 (1,64-3,23)

Table 2: Non-parametric univariate statistical comparison (Wilcoxon test) of endogenous steroid concentrations measured in the elite handball athletes (EFM) group before exercise, at maximum exercise and after exercise in the restitution phase during extreme mental exercise (n=40). Median values, interquartile ranges, the direction of the changes and the statistical significance of the differences are shown. ↑: increase; ↓: decrease; p≤0.05; **: p≤0.01; ***: p≤0.001. ALDO: aldosterone; ADRN: androstenedione; DHEA: dehydroepiandrosterone; DHES: dehydroepiandrosterone sulfate; 11DC: 11-deoxycortisol; OHPE: 17-α-hydroxypregnenolone; OHPG: 17-α-hydroxyprogesterone; CCON: corticosterone; CTOL: cortisol; CTON: cortisone; TEST: testosterone; DHTT: dihydrotestosterone; TEST/CTOL: testosterone-to-cortisol ratio; DHEA/CTOL: dehydroepiandrosterone-cortisol ratio; CCON/CTOL: corticosterone-cortisol ratio; CCON/DHEA: corticosterone-dehydro-epiandrosterone ratio.

	Baseline-maximum	Baseline-restitution	Maximum-restitution	pmol/mL		
	Response			Baseline	Maximum	Restitution
ALDO	↑ ***	↑ ***	↑ **	0.08 (0.08–0.10)	0.14 (0.08–0.32)	0.18 (0.08–0.40)
ADRN	↑ *	↑ ***		1.9 (1.1–2.7)	2.3 (1.4–3.2)	2.4 (1.7–3.4)
DHEA		↑ ***	↑ ***	10.3 (5.4–14.9)	11.7 (7.9–18.3)	18.7 (12.0–28.7)
DHES		↑ ***	↓ ***	6268 (5393–8622)	6702 (5895–9118)	6635 (5827–9348)
11DC		↑ **		0.14 (0.14–0.96)	0.32 (0.14–0.15)	0.46 (0.14–1.22)
OHPE		↑ **	↑ *	2.3 (1.7–4.8)	3.2 (1.9–5.2)	5.2 (1.9–8.5)
OHPG	↑ *		↓ **	1.9 (0.0–5.5)	2.5 (0.0–7.6)	1.8 (0.0–5.2)
CCON		↑ ***	↑ ***	9.5 (5.8–14.8)	10.7 (5.3–20.1)	26.9 (18.3–34.9)
CTOL		↑ ***	↑ ***	404 (325–484)	387 (293–483)	504 (415–567)
CTON	↑ ***	↑ ***	↓ *	82.2 (66.1–92.2)	100.2 (80.1–116.6)	84.4 (75.4–106.8)
TEST	↑ ***	↑ **	↓ **	19.8 (17.3–23.4)	25.1 (20.5–30.1)	21.9 (17.4–25.6)
DHTT	↑ ***	↑ **		2.3 (1.5–3.7)	2.6 (1.7–3.9)	2.6 (1.6–4.0)
TEST/CTOL	↑ ***	↓ **	↓ ***	0.05 (0.04–0.06)	0.06 (0.05–0.09)	0.04 (0.04–0.06)
DHEA/CTOL	↑ *	↑ ***	↑ *	0.03 (0.02–0.04)	0.04 (0.02–0.04)	0.04 (0.02–0.05)
CCON/CTOL		↑ ***	↑ ***	0.02 (0.02–0.04)	0.03 (0.02–0.04)	0.05 (0.04–0.07)
CCON/DHEA			↑ ***	0.91 (0.54–1.6)	0.90 (0.49–1.3)	1.4 (0.88–1.9)

4.3. Results of preliminary exercise-physiological tests

In the ET group, during both acute and long-term types of load, the DHES and DHTT concentrations significantly increased in the first phase of stress (baseline vs.

peak). There was a significant concentration decrease in CTOL (baseline vs. peak) and TEST (baseline vs. restitution).

During prolonged stress, nine steroids (DHEA, DHES, DHTT, 11DC, CCON, CTOL, CTON, OHPE, TEST) showed a significant increase in concentration, with each one being significantly higher at peak stress compared to the baseline. Compared to the peak of stress, three steroids (CTON, TEST, DHTT) showed a significant decrease in the restitution phase. The steroid concentrations measured during prolonged stress were significantly higher than those measured during acute stress, with these differences primarily observed at peak stress.

In the prolonged stress test, DHEA concentrations were significantly higher at baseline in comparison to the acute stress exercise. At peak stress, DHEA ($p \leq 0.05$*), 11DC ($p \leq 0.05$*), CTOL ($p \leq 0.05$*), and CTON ($p \leq 0.01$**) concentrations were significantly higher than during acute stress.

In the control group, the concentration of seven steroid hormones significantly increased due to stress. Except for DHTT, all steroid concentrations showed an increase compared to the baseline in the restitution phase. However, only one steroid (ALDO) had a significant increase. Most significant increases were observed at peak stress compared to baseline (ADRN, DHES, DHTT, CTON, OHPG, TEST). When comparing the control group and the ET group during acute stress, no significant differences were found.

5. Discussion

The continuous exposure of athletes to stress affects the entire body, including organs involved in steroidogenesis. Glucocorticoids and mineralocorticoids are necessary for normal blood pressure, diuresis, and the proper regulation of metabolic processes. When stress occurs, the HPA axis is activated, leading to an increase in the secretion of mineralocorticoids and glucocorticoids. However, the effects of hormones often manifest themselves later. In the stress state, the metabolic response that occurs is indeed a consequence of elevated steroid levels, with higher glucocorticoid levels resulting in more pronounced metabolic responses. In the stress response, the suppressive effect of glucocorticoids and their negative feedback regulatory effect prevent excessive response reactions. Steroid molecules originating from the adrenal cortex, gonads, and peripheral organs help the body adapt to physical and psychological stress.

There was no isolated change in the circulating concentrations of steroid hormones belonging to any group in any phase of stress, irrespective of the type of stress encountered. During extreme acute physical stress, metabolites of mineralocorticoids, glucocorticoids, and androgens all showed significant changes in response to stress, in contrast to extreme mental stress. In the case of physical stress, there were significantly more instances of substantial changes when comparing different stress phases in subjects. With one exception, all examined steroids showed a significant increase compared to the resting value during the restitution phase. In contrast, in the case of extreme mental stress, most significant changes were observable at the peak of stress compared to the resting value. These different changes in steroid hormone concentrations may perhaps be attributed to the minimal muscle work in the participants during mental stress.

Our results confirm that hormone secretion increases in response to physical stress, as observed in previous research, both for DHEA and DHES. Some studies show that while DHEA increases in men following exercise, DHES concentrations increase in women but not in men. In contrast, in our study, we detected an increase in both hormone concentrations in male athletes. In the case of CCON, the concentration changes were consistent with the literature. We were able to demonstrate an increase in serum concentrations of DHEA and CTOL hormones in response to acute physical stress in our study. We also confirmed an increase in

the concentration of TEST, alongside CTOL. The enzymes 3-(or 17-)beta-hydroxysteroid dehydrogenase (EC 1.1.1.51), 11β-hydroxylase (EC1.14.15.4), 17,20-lyase (EC 1.14.14.32, CYP17A1) and 21-hydroxylase (EC 1.14.14.16, CYP21A2), are all adrenocorticotropic hormone-regulated and are involved in the adrenal and gonadal steroidogenesis. Our results confirm that their activity increases in response to extreme physical stress. The functions of these enzymes are also associated with various pathological conditions. ALDO concentration determination is less common among the effects of the tested steroid hormones in response to stress, but our results show significant concentration changes during stress.

Throughout an athlete's career and during the preparation for their athletic career, it is essential to have accurate and comprehensive information about the athletes' health. Monitoring of physical and mental stress and rest periods is crucial in the early identification of athletes at risk of developing overtraining syndrome. The methodology we applied can assist in monitoring the endocrine homeostasis of athletes and in characterizing their responses to stress. Profiling endogenous steroids appears to be useful in assessing the stress tolerance of athletes preparing for their athletic careers. Characterizing the steroid homeostasis can help optimize their physical stress and serve as a reference for evaluating the effects of future events and physiological changes on their stress tolerance. The results may facilitate the identification of biomarkers suitable for monitoring the steroid homeostasis of athletes under regular physical stress.

6. Conclusion

In our study, we were the first to apply steroid profiling methodology to characterize the response of elite athletes to physical and mental stress until exhaustion. Furthermore, we were the first to apply steroid profiling methodology to elite athletes for a 2-hour, moderate-intensity, continuous stress test based on maximal effort. The profiling approach we used allowed for the comprehensive characterization of adrenal cortex and gonadal steroidogenesis shortly after sample collection, in response to extreme physical and mental stress. It is particularly important for athletes to consider the responses elicited by extreme physical stress, not only their immediate effects but

also potential long-term consequences. Athletes often train multiple times a day, frequently for years, which constitutes chronic stress. Therefore, continuous monitoring and screening examinations are essential for them.

7. New scientific results

1. The quantity of steroid hormones produced in the adrenal cortex and gonads, which we examined, showed a significant increase during at least one phase of extreme acute stress.
2. The most significant changes in steroid hormone concentrations in elite athletes were observed during the restitution phase following extreme acute physical stress.
3. Extreme physical stress simultaneously activated the mineralocorticoid, glucocorticoid, and androgen pathways. The significant changes in steroid concentrations in the examined subjects indicate a complex and intense physiological response. The variation in the concentrations of circulating adrenal, gonadal, and peripheral steroids did not suggest selective activation of steroid-producing organs.
4. In addition to what has been described in the literature, we were able to confirm changes in the concentrations of other steroid hormones (ADRN, CCON, DHTT, OHPE) in elite athletes in response to extreme physical stress.

8. Acknowledgement

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9. Publication list

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Csöndör, É.; Karvaly, G.; Ligetvári, R.; Kovács, K.; Komka, Zs.; Móra, Á.; Stromájer-Rácz, T.; Oláh, A.; Tóth, M.; Ács, P. Adrenal, Gonadal and Peripherally Steroid Changes in Response to Extreme Physical Stress for Characterizing Load Capacity in Athletes. Metabolites. 2022, 12: 2 P: 91, 11 p.
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7. sz. melléklet

DOKTORI ÉRTEKEZÉS BENYÚJTÁSA ÉS NYILATKOZAT A DOLGOZAT EREDETISÉGÉRŐL

Alulírott

név: Csöndör Éva

születési név: Csöndör Éva

anyja neve: Zsigrai Erzsébet

születési hely, idő: Nagyatád, 1991.05.22.

Az extrém fizikális és mentális terhelés, valamint az endogén szteroid bioszintézis összefüggései professzionális sportolóknál című doktori értekezésemet a mai napon benyújtom a Pécsi Tudományegyetem Egészségtudományi Doktori Iskola 7. Program (PR-7: Sport és Egészségtudomány), S-26 (A kardiorcspiratórikus rendszer szabályozása megnövekedett fizikális és pszichés terhelés alatt) Programjához/témacsoportjához

Témavezető(k) neve: Dr. Oláh András, Prof. Dr. Tóth Miklós

Egyúttal nyilatkozom, hogy jelen eljárás során benyújtott doktori értekezésemet
- korábban más doktori iskolába (sem hazai, sem külföldi egyetemen) nem nyújtottam be,
- fokozatszerzési eljárásra jelentkezésemet két éven belül nem utasították el,
- az elmúlt két esztendőben nem volt sikertelen doktori eljárásom,
- öt éven belül doktori fokozatom visszavonására nem került sor,
- értekezésem önálló munka, más szellemi alkotását sajátomként nem mutattam be, az irodalmi hivatkozások egyértelműek és teljesek, az értekezés elkészítésénél hamis vagy hamisított adatokat nem használtam.

Dátum: 2023. 09. 18.

Csöndör Éva
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doktorjelölt aláírása

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témavezető aláírása

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társtémavezető aláírása